Rapid Detect-Identify-Decontaminate Kit for Biological Agent Hazard Mitigation in Aircraft Interiors

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Introduction

In the aftermath of a biological attack, aircraft decontamination would be a complex, challenging process. The Edgewood Chemical Biological Center (ECBC) has set out to develop technologies for effective aircraft decontamination without adversely affecting the aircrafts' sensitive equipment and electronics. Current biological decontamination systems for aircraft rely mostly on thermal decontamination and vaporized hydrogen peroxide (VHP). However, a number of challenges remain to be resolved. For example, the ability of VHP to penetrate into occluded spaces, and the maximum acceptable temperature for neutralizing spore-forming bacteria without adversely affecting the aircraft electronic and mechanical systems in the cabin and cargo areas are unknown. Current tactics, techniques and procedures (TTPs) in the Air Force Manual (AFMAN) 10-2503 cite the use of a wet/damp cloth to wipe surfaces for spot-decontamination of suspected biological agents. Assurance of decontamination efficacy for certain biological agents, such as spores, has been shown to be marginal. The Joint Platform Interior Decontamination (JPID) and Joint Service Sensitive Equipment Decontamination (JSSED) programs were launched to address hazard mitigation of sensitive equipment but were cancelled due to unsustained funding. As a result biological decontamination for aircraft electronic and mechanical systems remains a technical gap in the hazard mitigation arena.

Relevance

This project explored an approach for rapid detection and decontamination of aircraft interiors using a kit designed for sampling aircraft interior, detection of spore-forming bacteria and decontamination of suspected areas. The kit includes Government-Off-the-Shelf (GOTS) Hand-Held Assays (HHAs) used for rapid presumptive identification of *Bacillus thuringiensis* spores prior to conducting decontamination operations and after mitigation efforts. ECBC's C-130 cargo aircraft provided the test bed for the rapid detection and decontamination kit testing, and ECBC's barcoded *B. thuringiensis* spores were used as the simulant for contamination of the aircraft surfaces. The decontaminant candidate selected was the Surface Decontaminating Foam (SDF). Proof-of-concept for this decontamination method resulted in a conceptual rendering of a rapid detnection and decontamiation kit for aircraft hazard mitigation with potential for development of a prototype kit in future studies.

Materials and Methods

Microbial Pathogen and Culture Conditions. Barcoded *Bacillus thuringiensis* kurstaki spores (BTK, ECBC, Biosciences) were prepared in Phosphate Buffer Saline + 0.1% Triton X-100 (PBST) at approximately 4.0E+08 CFU/ml. The spores were cultured on Trypticase Soy Agar (TSA) plates in triplicate from 10^{-1} to 10^{-6} serial dilutions and incubated for 16 hrs at 37° C. Replicate dilutions generating 20 – 200 colonies were computed and averaged to derive the mean log colony forming units.

Aircraft Test Surfaces. Two military-relevant aircraft surfaces were identified for sampling in the cargo portion of the C-130 aircraft: aluminum and non-skid. The aluminum and non-skid test areas were taped-off rectangular sectors 6.3 x 8.5 in (53 cm^2) that were divided into 4 equal quadrants for pre-decontamination (Pre 1A, 1B) and post-decontamination (Post 2A, 2B) sampling.

Contamination. Each test area was contaminated by dispensing 1 milliliter (ml) of BTK spore stock (4.0E +08 CFU/ml) in twenty 50 µl droplets (5 per quadrant) on the test area. The spores were allowed to dry overnight at ambient temperature for sampling the following day.

Decontamination. Allen-Vanguard's (Ontario, Canada) Surface Decontaminating Foam (SDF) was used to decontaminate the aircraft test surfaces contaminated with BTK. The SDF decontamination foam consisted of a decontamination powder (GPA-2100) that was mixed separately with water and added to the liquid foaming agent (GCE-2000 and GPD-2100, surfactant). The decontaminant foam was sprayed on the contaminated test surfaces and allowed to dwell for 30-min.

Sampling. Pre-decon and post-decon samples were collected from each test surface by swabbing the respective quandrants and performing HHA assessments on-site, followed by colony culture and polymerase chain reaction (PCR) in the laboratory. Sampling occurred over a 3-day period with a total of six replicate data points.

Hand-Held Assays. *B. thuringiensis* kurstaki*/B. globigii (*BTK/BG) Hand-Held Assays (HHA) were purchased from the Chemical Biological Medical Systems (CBMS) Critical Reagents Program (CRP) for presumptive identification of BTK pre- and post-decontamination presence on aluminum and non-skid aluminum test surfaces.

PCR Quantification. BTK PCR reactions were performed in 20 µL volumes in 384-well optical PCR plates (Cat. 4309849; Applied Biosystems, Foster City, CA). Each reaction was set up using 14.75 µL of master mix, 0.25 µL of Taq polymerase, and 5 µL of extracted DNA product. The thermocycler conditions were as follows: 50°C for 2 minutes, 95°C for 20 seconds, 40 cycles of 95°C for 1 second, and 60°C for 20 seconds. Analysis for all assays was performed using Sequence Detection Software v.2.3. Detection was defined as a Ct value less than 40 with the threshold 2 standard deviations above background.

Abstract

We demonstrated the Proof-of-Concept for Aircraft Biodetection and Decontamination kit. We utilized C-130 aircraft as a test bed to evaluate the efficacy of Surface Decontamination Foam (SDF) technology against bacterial spores on different material surfaces that are found in aircraft interiors. Barcoded spore-forming bacteria (*Bacillus thuringiensis* kurstaki, BTK) served as a simulant for *B. anthracis*. The spores were deposited on the test surfaces at a concentration of 4.0E+08 CFU/53.5 cm^2. The contaminated surfaces were decontaminated with SFD for 30-minutes. The decontamination efficiency was determined by measuring the pre- and post-decontamination levels of the spores using Hand Held Assays and PCR. SDF decontaminant achieved a 7-log reduction and zero (0) residual threshold. This approach can be applied to real world situations such as, cases where a commercial jet liner is exposed to natural or deliberate disease pathogens. Therefore, a conceptual rendering of the kit was developed. The kit includes government-off-the-shelf Hand-Held Assays for rapid pathogen identification, as well as decontamination and personal protection components.

Figure 1. Conceptual rendering of Aircraft Biodetection & Decontamination Kit.

Results

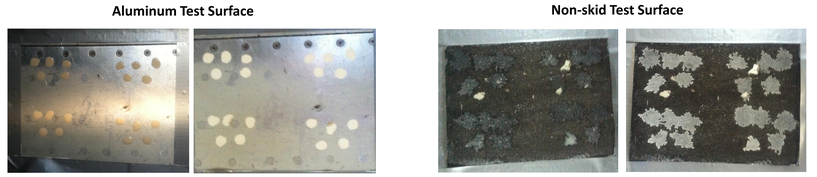

Figure 2. Photos of BTK wet and dried spores on aluminum (1A & 1B) and non-skid (2A & 2B) aircraft test surfaces.

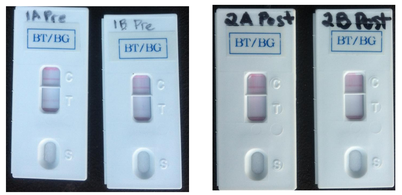

Figure 3. BTK HHA tickets showing pre-decontamination presumptive identification of BTK spores deposited on an aircraft aluminum test surface at a target challenge of 4.0E+08 CFU (left) and BTK post-decontamination HHA assessment (right).

Results (cont'd)

Table 1. Log_{10} values of the number of *B. thuringiensis* colonies for pre-decon and post-decon test samples using SDF decontaminant.

Test Date	Sample ID	Aluminum Log_{10} CFU		Non-skid Log_{10} CFU	
		Pre-Decon	Post-Decon	Pre-Decon	Post-Decon
8/14/13	Swab 1	8.4	0	7.7	0
	Swab 2	8.4	0	7.7	0
8/27/13	Swab 1	8.6	0	8.6	0
	Swab 2	8.6	0	8.1	0
8/28/13	Swab 1	8.6	0	8.2	0
	Swab 2	8.6	0	8.3	0

Table 2. PCR Ct values for *B. thuringiensis* colonies pre-decon and post-decon test samples using SDF decontaminant.

Test Date	Sample ID	Aluminum Ct Value		Non-skid Ct Value	
		Pre-Decon	Post-Decon	Pre-Decon	Post-Decon
8/14/13	Swab 1	27.88	0	33.86	0
	Swab 2	27.70	0	32.76	0
8/27/13	Swab 1	23.92	0	26.62	0
	Swab 2	24.69	0	26.12	0
8/28/13	Swab 1	23.55	0	25.78	0
	Swab 2	24.46	0	25.33	0

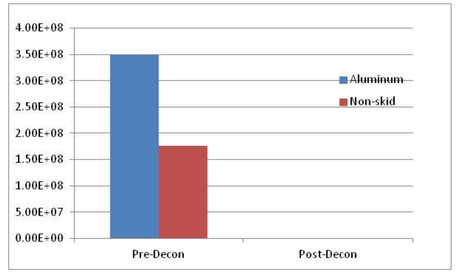

Figure 4. Graphical representation of BTK CFU recovery for pre- and post-decontamination efficacy testing on aluminum and non-skid C-130 test surfaces using a 30-min dwell time for SDF decontaminant (n = 6 replicates).

Discussion

This project explores an approach for rapid detection and decontamination of aircraft interiors using a kit designed for sample collection, detection and decontamination of suspected areas. The kit includes GOTS Hand-Held Assays used for rapid identification of *Bacillus thuringiensis* spores before and after decontamination. When evaluated with SDF decontamination technology on C-130 cargo aircraft, a 7-log reduction of *B. thuringiensis* spores was achieved, with no residual spores detected on the decontaminated surfaces. The entire kit weighs approximately 10 pounds and contains all reagents and supplies for performing 20 tests. The entire assay takes approximately 2 hour, including sample collection, decontamination and detection, and is performed by one operator.

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